Wnt Signaling Is Required for the Maintenance of Human Limbal Stem/Progenitor Cells In Vitro.

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Public Summary:
Purpose: A chemical approach to examine the role of Wnt signaling in maintaining the stemness and/or proliferation of limbal stem/progenitor cells (LSCs). Methods: LSCs were isolated from human donor eyes and cultured as single cells for 12 to 14 days with the following small molecules: IIIC3, an antagonist of the Wnt signaling inhibitor Dickkopf (DKK), and IC15, a Wnt signaling inhibitor. Proliferation of LSCs in the presence of IIIC3 and IC15 was determined by the number of cells and colonies established. Maintenance of stemness was determined by p63alpha, cytokeratin (K)12, and K14 expression. Results: Activation of Wnt, through IIIC3-mediated DKK inhibition, resulted in similar colony forming efficiency (CFE) as in the untreated LSCs, but significantly increased the number of cultivated cells 7.21% with 5 muM. Inhibition of Wnt with IC15 significantly reduced the CFE (P </= 0.01) and the number of cultivated cells by 16% to 29%. Percentage of cells expressing high levels of p63alpha (p63alphabright) and quantity of small cells (</=12 mum), which contain the LSCs, increased 4.71% and 11.26% (both P < 0.05), respectively, with 5 muM IIIC3. All concentrations of IIIC3 and IC15 retained the K14 undifferentiated marker (97%), while differentiation, as detected by expression of K12, was found in up to 2% of cells in 1 muM IIIC3, 1 muM IC15, or 5 muM IIIC3. Conclusions: Wnt signaling is required in LSC proliferation and maintenance of an undifferentiated state. The current study is a proof of concept that the Wnt pathway could be modulated in LSCs to enhance or decrease the efficiency of human LSC expansion.

Scientific Abstract:
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